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Award Number: W81XWH-04-1-0242

TITLE: Therapy Selection by Proteomic Profiling

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REPORT DATE: February 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 01-02-2007		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 26 Jan 2006 – 25 Jan 2007	
4. TITLE AND SUBTITLE Therapy Selection by Proteomic Profiling				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-04-1-0242	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Simon W. Hayward, Ph.D. E-Mail: simon.hayward@vanderbilt.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Vanderbilt University Medical Center Nashville, Tennessee 37203-6917				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The long term goal of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The central hypothesis of this proposal is that a subset of the proteins expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The purpose of this work is to generate predictive methods which will allow patients to be selected for specific treatment protocols. In the current funding period we have completed the collection of human prostate cancer tissue, its grafting to mice and treatment of these mice with Taxotere. Tissues have been harvested and MALDI-MS profiles generated from both tumor epithelium and adjacent stroma. Efforts are currently underway to rigorously define and quantitate the response to Taxotere of the tissue samples, on an individual basis, prior to initiation of bioinformatic analysis of the mass spectrometry data sets. The project is proceeding behind its predicted timeline as outlined in the accepted statement of work and a no cost extension to complete the work has been requested.					
15. SUBJECT TERMS proteomic profiling, taxotere, prediction of response, biostatics					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER (include area code)

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Introduction

The **long-term goal** of this work is to develop a new prognostic tool with which to determine the response of a patient to a given therapy, with the view of providing the most appropriate treatments tailored to individual patients. The **central hypothesis** of this proposal is that a subset of the genes expressed in a prostate tumor can be used to predict response to specific therapeutic regimens. The **purpose** of this work is to generate predictive methods that will allow patients to be selected for specific treatment protocols. The **rationale** is to utilize a novel method of human prostate cancer tissue xenografting in combination with state of the art proteomic, biostatistical and bioanalytical analysis to generate new prognostic tools. This project is an essential “proof of principle” step in the sense that if this methodology is successful with Taxotere it should be applicable to any new therapeutic approach that exists or which will be developed in the future. This work will allow us to design new predictive proteomic assays to determine whether specific patients will respond to Taxotere. This project is linked to, and shares tissue resources with a related study DAMD 17-03-1-0047 which has similar aims in terms of gene expression. The funding of these two projects running in parallel with the same patient samples allows the possibility of mixed genomic/proteomic-based tool development.

Statement of Work

PCRP Idea Development Award

W81XWH-04-1-0242

Therapy Selection by Proteomic Profiling

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Task 1 Task 1 is now completed.

Generate Matrix Assisted Laser Desorption Ionization –Mass Spectrometry (MALDI-MS) profiles for prostate cancer samples from 260 patient tumor samples

- a) As cases present, collect 260 histopathologically-confirmed prostate cancer tissue cores. Snap freeze core fragments
- b) Cut adjacent frozen sections onto slides and onto MALDI-MS target plates; identify areas of tumor by hematoxylin and eosin (H&E) staining (months 1-25)
- c) Run MALDI-MS analysis of 260 samples, store data sets electronically (months 1-25)

Task 2. Task 2 is now completed

- a) Graft tissues from the cores used in task 1 to pairs of Severe Combined Immune Deficient (SCID) mice (months 1-24)
- c) Treat one of each pair of mice with Taxotere (months 1-25)
- d) Sacrifice mice and harvest tissues. (months 2-26)
- e) Perform Terminal Nuclear End Labeling (TUNEL) and histopathologic analysis of both control and Taxotere-treated samples. Calculate apoptotic indices (months 3-28).

Task 3 Status – ongoing (see discussion in report.)

Biostatistical analysis (to be performed by the VICC Biostatistics Core).

- a) Identification of protein expression profiles which predict histopathologic response to Taxotere. Biostatistical analysis to determine a protein expression profile in tissue cores which predict histopathologic response to Taxotere in a xenograft model (months 26-32).
- b) Identification of proteins regulated by Taxotere in responsive and non-responsive tissues. Biostatistical and bioinformatic analysis will be used to identify protein peaks regulated by Taxotere in responsive and non-responsive tissue samples (months 28-34).

Task 4 Status, not yet started

Prediction of response of patients in a clinical trial setting (months 35-36)

Based upon MALDI-MS analysis of archived snap frozen tissue the ability of the protein expression patterns identified in task 3 to predict response in a clinical trial will be tested. Run MALDI-MS analysis on samples; predict response based upon data acquired in earlier tasks. Test results by breaking patient code and correlating actual and predicted responses.

Summary of the Project
Work Ongoing and Completed
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We have requested a no cost extension of this project to allow time to troubleshoot the next stage of analysis and to complete the work in task 3, once completed this should allow us to move to task 4.

Since the second annual report we have been able to complete the collection and processing of tumor samples through mice. Grafts have been harvested and MALDI-MS profiles of both tumor epithelium and adjacent stromal cells collected separately. This effectively completes the work outlined in tasks 1 and 2. The MALDI-MS profiles are now electronically stored and represent one of the key data sets which was to be generated from this project.

The major problem encountered in this period has been interpretation of the response of tissues to Taxotere. We initially proposed using apoptosis as our primary reporter of response to Taxotere. This method proved effective in preliminary studies. However a complete analysis of the full data set showed a disappointingly low level of apoptotic response in the treated tissues, and perhaps more worrying a level of false positive results which could compromise the data analysis. As noted in the previous annual report, we established a collaboration with Dr. Wang's laboratory in Vancouver. However, given the small size of the grafted tissue fragments, histopathologic examination has not proven to be a useful method to accurately determine response in a significant proportion of tissues.

Since the statistical analysis is an expensive item which will be charged whether or not interpretable data results, we wish to be in the best possible position to gain useful data at this stage of analysis. We are therefore undertaking analysis of a number of other surrogate markers of response to this drug. Specifically at this point, we are examining the expression of a series of markers using immunohistochemical staining of sections. The candidate molecules being examined are:

Thymidine phosphorylase, which is induced in a number of tumor types following Taxotere treatment, typically 5-10 fold. Increased levels are long-lasting (peak levels >10 days). Controversial as a predictor for Taxotere response in a number of cancers, but should be useful to confirm exposure of xenografts to therapeutic levels of Taxotere by comparison of treated and non-treated patient matched samples.

Anti- α -Glu-tubulin detects stabilized detyrosinated (Glu) microtubules, which are increased with Taxotere treatment. This marker can be used in conjunction with thymidine phosphorylase levels to confirm exposure of tumor xenografts to therapeutic levels of Taxotere, but may also be useful for detection of resistant tumor cells, as resistant cells often have beta-tubulin mutations that prevent microtubule stabilization.

Tubulin stabilization, which is a definitive positive response to Taxotere is also being examined to determine which tumors contain cells in which microtubule structure has been locked, the consequence of successful

The elevated expression of genes controlling the cellular redox environment has been implicated in Taxotere resistance in breast tumors. Iwao-Koizumi, et al. found that high pre-treatment levels of thioredoxin, glutathione-S-transferase pi 1, and peroxidoxin 1 in breast tumor biopsy samples correlated with resistance to Taxotere response in patients. They also demonstrated that overexpression of these genes protected cultured mammary tumor cells from induced-induced cell death, suggesting that enhancement of the redox system plays a major role in Taxotere resistance. The expression levels of these genes, as measured by microarray analysis in a related study (DAMD 17-03-1-0047), were found to range from very high to very low, and may be useful for predicting resistance to Taxotere in prostate cancer.

At the time of writing we have optimized staining for thymidine phosphorylase, preliminary staining runs to analyze microtubule stabilization have also been performed. Optimization of a profile of these markers should allow us to establish a rigorous profile of response to Taxotere in our samples thus allowing us to move to the bioinformatic analysis with increased confidence in the validity of the data which will emerge from such an analysis.

Personnel Changes

None since last report

Research Goals/Accomplishments

- Task 1 completed.
- Task 2 completed.
- TUNEL staining and analysis of apoptotic indices completed, analysis of the data generated by this process suggest an unacceptably high level of false positive results.
- A revision of the method for assessing response to Taxotere is being pursued in order to provide the most rigorous possible basis for subsequent bioinformatic analysis.

Reportable Outcomes.

None

Conclusions.

This work continues to proceed behind the predicted timeline. As previously noted, the major limitation has been availability of prostate samples. Technical improvements in bioinformatic analysis which have occurred in the period since the planning of this proposal improve the power of analysis, however at this point the most critical problem is accurate determination of response to Taxotere. As noted above we are exploring new methods to approach the quantification of this response before moving to the data analysis stage. The overall aims and long-term goal of the proposed work remain unchanged.